lipid bilayer to protect and exchange their contents with the cell. We have developed methods primarily based on atomic force microscopy that allows precise investigation of the mechanical properties of liposomes and that could be applied to study other related organelles/viruses. The mechanical properties of small, spherical vesicles were probed by applying very low forces (~ 0.1 nN), which led to a maximum 10 % deformation. The effects of lipid composition, temperature, osmotic pressure and the radius of curvature were studied for liposomes with diameters between 30 and 150 nm. The liposome deformation was modeled using finite element methods in order to extract the lipid bilayer elastic properties. For the larger liposomes we find a very good agreement with previously reported experiments on micrometer sized giant vesicles.

3071-Pos

Probing the Mechanical Properties of Single Scleroproteins with Optical Tweezers

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Stretching and relaxing single proteins provides quantitative information on their elasticity and other mechanical properties. This can be done with optical tweezers, a technique in which the ends of the protein are chemically attached to micron-scale spheres, used to manipulate the protein and measure its response. We are working on the application of this technique to scleroproteins, nonglobular proteins whose mechanical properties are of direct relevance to their physiological roles. These proteins self-assemble into hierarchically organized load-bearing structures, often found in the extracellular matrix. The ability of optical tweezers to manipulate single molecules and higher-order structures suggests their application to probing the mechanical response at different hierarchies of assembly. Applying this technique to stretch these single proteins presents many challenges, including the production of constructs with appropriate labels for attachment to microspheres, relatively short contour lengths which can introduce experimental artifacts, and self-aggregation and binding interactions of these predominantly insoluble proteins, which make it difficult to isolate and manipulate single molecules. We discuss our work to overcome these challenges, with a specific focus on elastin.

3072-Pos

Elastic Behavior of ssDNA in Salty Solutions

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The interaction between highly charged poly-ions, such as DNA, and the smaller ions in salty solutions is of fundamental importance to the basic processes of molecular biology (e.g., ion-mediated nucleic acid folding, collapse and stabilization of proteins). Despite its importance, this phenomenon is poorly understood, particularly for multivalent ions where mean-field theories (e.g. Debye-Huckel) break down. By stretching single denatured ssDNAs in monovalent salt solutions, we have established that force-extension measurements directly and quantitatively probe electrostatic effects on charged polymers in solution (O.A. Saleh *et al.*, PRL **102**, 068301 (2009)). We exploited access to the 'tensile blob' regime to show that, for a broad range of NaCl concentrations, ssDNA behaves as a real polymer in good solvent with a Kuhn length linearly proportional to the Debye length. Here, we present data on the effects of cations with different valences and chemistries on ssDNA structure. We find that the effects of divalent ions greatly exceed those predicted by simple Debye-Huckel calculations and discuss our data in the context of more realistic theories.

3073-Pos

Mechanical Unfolding of Cardiac Myosin Binding Protein-C by Atomic Force Microscopy

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Cardiac myosin binding protein-C (cMyBP-C) is a member of the immunoglobulin (Ig) superfamily of proteins and consists of 8 Ig- and 3 fibronectin (Fn)-like domains along with a unique regulatory sequence referred to as the M-domain. Domains near the C-terminus bind tightly to myosin and mediate the association of MyBP-C with thick (myosin-containing) filaments, whereas N-terminal domains of MyBP-C, including the M-domain, bind reversibly to myosin S2 and/or actin. The ability of MyBP-C to bind to both myosin and actin raises the possibility that MyBP-C cross links thick and thin (actin-containing) filaments and thereby imposes a drag that regulates shortening velocity during contraction. To investigate the mechanical properties of the proposed thick-thin filament linkage, we used atomic force microscopy (AFM) and electron microscopy (EM) to assess the single molecule elasticity and mechanical stability of full-length mouse cardiac (c) MyBP-C expressed in sF9 cells. Force-extension curves showed that cMyBP-C is extensible via unfolding of individual domains evident as "saw tooth" peaks in force spectra. Spectra with up to 12 peaks were obtained. The force required to unfold the domains varied, with the least and most stable domains unfolding at forces <50 pN and >100 pN, respectively, suggesting that a mechanical hierarchy exists along cMyBP-C. EM images of purified, rotary shadowed cMyBP-C showed that molecules were frequently V- or U-shaped with lengths ~44 nm. These data indicate that cMyBP-C is extensible and contains regions with variable resistances that could slow sarcomere shortening or limit lattice expansion. Supported by NIH HL080367.

3074-Pos

Active Force Clamp Control of Optical Tweezers

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In a typical high-resolution optical tweezers (OT) experiment a molecular motor changes the contour length of a trapped dumbbell-construct. Unless the inter-trap distance is actively controlled the OT increases the load on the molecular motor as it steps along the template. To counter this phenomenon we implement a real-time controller for the OT to be used in constant force measurements.

We trap a dumbbell construct (bead-DNA-bead) in an inverted microscope by dividing a CW laser beam into a stationary trap and a steerable trap. Separate low power detection lasers and position sensitive detectors in the back-focal plane measure the position of both beads. The position of the bead in the stationary trap is used for constant-force feedback control. The feedback algorithm runs a Proportional-Integral-Derivative-controller on a field programmable gate array, and acousto-optical deflectors update the steerable trap position at a rate of 200 kHz.

We test the force clamp control with a 10kb dsDNA molecule and present a theory explaining the power spectrum of the force clamped bead's position. We study the effect of controller bandwidth by digitally filtering the signal used for feedback control, and test the response time of our real-time controlled optical tweezers with a RNA hairpin opening/closing reaction.

3075-Pos

Protein-DNA Interactions Studies using Magnetic Tweezers

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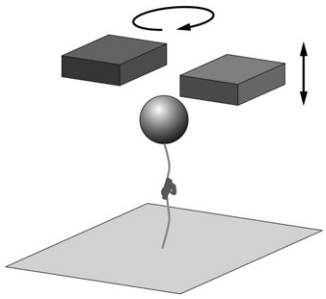
Single molecule force spectroscopy techniques allow the forces, energy barriers, and mechanisms of biologically important structural transitions to be manipulated and observed at the single molecule scale. A major advantage of magnetic tweezers is the ability to manipulate not only the force in such systems, but also the torque, which is extremely important in processes involving the DNA double helix, since such processes frequently involve rotational motion. We will present recent results studying protein-DNA interactions using a newly constructed magnetic tweezer.



3076-Pos

Single-Molecule Atomic-Force Spectroscopy Captures a Novel Class of Molecular Nanosprings with Robust Stepwise Refolding Properties

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Biological systems are constantly under mechanical stress either during movement or when acted upon by external forces. The identification of proteins motifs that behave as biological springs will be important for understanding how cells respond to mechanical stimuli and can also propel the design of non-biological nanomaterials. We report here identification of a large class of alpha-helical spiral or solenoid-shaped proteins comprised of ANK-R, ARM, or HEAT repeats that rapidly and forcefully refold following stretching. Each of these repeats unfolds and refolds in equilibrium through discrete events involving

individual repeats or their alpha-helical subunits. We also present evidence for the capture of unfolding/refolding transient events while stretching or relaxing by AFM and analyze differences in refolding lengths and forces for each repeat. This class of stacked helical-repeats behave as molecular nanosprings, are likely important for cellular mechanosensation, and can be used as platforms for structural elements of nanomechanical systems based on proteins. Supported by the NIH (PEM) and HHMI (VB).

3077-Pos

High Force Elastic Profiles of Single and Double Stranded Polynucleotides Probed with AFM Force Spectroscopy

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Elasticity is an important property of nucleic acids. During cellular processes, DNA and RNA are subjected to various mechanical forces which greatly deform their original structure. Also, in the field of DNA nanotechnology, an understanding of how DNA will react to mechanical loading will allow for the design of novel nanostructures with different forms and functions. The elastic response of nucleic acids subjected to very high loadings on the order of 1 nN has not been previously studied. We use AFM-based single-molecule force spectroscopy to, for the first time, compare and contrast the elasticity of different sequences of double and single stranded polynucleotides, including single stranded poly(A), poly(dA), poly(dT), poly(C), and poly(dC); and double stranded poly(dA)poly(dT), poly(dA-dT), poly(dG)poly(dC), and poly(dG-dC). We found that even up to forces as high as 800 pN poly(dA) is stiffer than the other single stranded structures. We have also observed marked differences in the behavior of double stranded poly(dA)poly(dT) and poly(dG)poly(dC) with poly(dA-dT) and poly(dG-dC), respectively. Despite their different elasticities, these double stranded polynucleotides exhibit striking features similar to those exhibited by poly(dA) when stretched. We investigate the origin of these differences and similarities in terms of base-base and base-backbone interactions.

3078-Pos

Adhesion Mechanisms of the Mussel Foot Proteins mfp-1 and mfp-3

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Mussels adhere to a variety of surfaces by depositing a highly specific ensemble of 3,4-dihydroxyphenyl-L-alanine (DOPA) containing proteins. The adhesive properties of Mytilus edulis foot proteins mfp-1 and mfp-3 on mica (a common alumino-silicate clay mineral) and TiO2 surfaces were directly measured at the nano-scale by using a surface forces apparatus (SFA). The adhesion energy between mfp-3 and mica was on the order of W=3×10-4 J/m2 which corresponds to an approximate force per plaque of ~100 gm - more than enough to hold a mussel in place if no peeling occurs. In contrast, no adhesion was detected between mica surfaces bridged by mfp-1. AFM imaging and SFA experiments showed that mfp-1 can adhere well to a single mica surface, but in order for bridging to occur between two mica surfaces the protein must be sheared or allowed extended contact time with the opposing surface. On TiO2 surfaces the mfp-1 interaction is 10-fold stronger than with mica, presumably due to capability of DOPA to form coordination bonds with the TiO2 surface. The results are consistent with the apparent function of the proteins, i.e., mfp-1 is disposed as a "protective" coating and mfp-3 as the adhesive or ''glue'' that binds mussels to surfaces. While mussel foot protein is capable of making strong adhesive bonds with TiO2, the adhesion to mica is actually weak and likely due to weak physical interactions rather than chemical bonding. However, strong adhesion forces of mussel plaques can arise as a consequence of plaque geometry (i.e., their inability to be peeled off) even on surfaces such as mica that do not have a high intrinsic surface or adhesion energy, W.

3079-Pos

Motor-Substrate Interactions in a Ring ATPase

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Many processes in biology, including DNA recombination, prokaryotic cell-segregation, gene transposition, and viral DNA packaging, involve the translocation of DNA or RNA by ATP-driven ring motors belonging to the ASCE/AAA superfamily. While the mechanism by which these motors convert the chemical energy from ATP hydrolysis to mechanical work is beginning to be understood, little is known about how these motors engage their nucleic acid substrates. Do motors contact a single DNA element, such as a phosphate or a base, or are contacts distributed over multiple parts of the DNA? In addition, what role do these contacts play in the mechanochemical cycle? Here we use a single-molecule assay for the genome packaging motor of the *Bacillus subtilis* bacteriophage phi-29 to address these questions. The full mechanochemical cycle of the motor involves two phases–an ATP loading dwell followed by a translocation burst of four 2.5-bp steps. By challenging the motor with a variety of modified DNA substrates, we show that during the dwell phase important contacts are made with adjacent phosphates every 10-bp on the 5'-3' strand in the direction of packaging. In addition to providing stable, long-lived contacts, these phosphate interactions also regulate the chemical cycle. In contrast, during the burst phase, we find that DNA translocation is driven against large forces by extensive contacts, some of which are not specific to the chemical moieties of DNA. Such promiscuous, non-specific contacts may reflect common translocase-substrate interactions for both the nucleic acid and protein translocases of the ASCE superfamily.

3080-Pos

Mapping Micro-Mechanical and Micro-Structural Changes in the Ageing Aorta

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In healthy individuals, arterial function is critically dependent on the biomechanical properties of stiff fibrillar collagens, resilient elastic fibre proteins and contractile smooth muscle cells. Although age-related reductions in arterial compliance (arteriosclerosis) are associated with chronic hypertension and hence with the development of aortic aneurysms, heart failure and stroke, the differential role played by each of these vascular components in the progression of disease remains unclear.

The scanning acoustic microscope (SAM), when operated at frequencies close to 1GHz, is capable of measuring acoustic wave speeds (which are related to tissue stiffness) with a spatial resolution of ~1 micrometer. Using unfixed tissue cryo-sections, we mapped variations in wavespeed from the intimal surface of young (less than 1.75years) and old (more than 8.00years) sheep aortas. Whilst there was a significant age-related increase in mean wavespeed, across the tissue (young: 1.847km/s, SEM 0.004km/s; old: 1.882km/s, SEM 0.003km/s; Mann Whitney U test, $p<0.001$) the increase was most pronounced in the inter-lamellar (IL) regions located between large elastic lamellae (EL) (wavespeed increase; IL: 0.047km/s, EL: 0.021km/s). Atomic force microscopy of ovine aorta cryo-sections identified both fine elastic fibres and collagen fibril bundles within this IL space. Collagen and elastin contents of young and old aortas were determined (as a percentage of tissue section area) using light microscopy of picrosirious red and Miller's stained sections respectively. Although collagen content increased significantly in old compared with young sheep (young: 30.97%, SD 2.63%; old: 44.86%, SEM 5.00%; Student's t-test $p<0.05$) there was no significant change in elastin content (young: 49.75%, SD 4.86%; old: 49.98%, SEM 4.27%; Student's t-test $p=0.97$).

These observations suggest, therefore, that gross mechanical stiffening of the ageing aorta, may occur primarily as a result of localised collagen remodelling in the space between elastic lamellae.

3081-Pos

Integrating Dynamic Force Spectroscopy and Surface Plasmon Resonance to Define the Energy Landscape for Integrin:Ligand Binding

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Background: Blood clots, aggregates of platelets trapped in a mesh of fibrin fibers, can impede normal blood flow, causing heart attacks and strokes. Therapeutic interventions use drugs with Arg-Gly-Asp (RGD) sequences to disrupt interactions between platelet αIIbβ3 integrins and the fibrin network's subunits. Determination of the αIIbβ3-ligand energy landscape will elucidate the successes and limitations of integrin antagonists.

Objectives: Integrating surface plasmon resonance (SPR) and dynamic force spectroscopy (DFS), we studied the energetics of αIIbβ3: ligand interactions. We focused on cHArGD, a cyclic peptide structurally similar to eptifibatide, a cardiovascular disease drug, as well as to fibrinogen's KQAGDV integrin-recognition sequence.

Methods: DFS determined single bond rupture forces, the dissociation constant k_{off}, and the rupture distance x_{-1} for αIIbβ3: cHArGD interactions. SPR determined the kinetic and thermodynamic parameters for αIIbβ3: cHArGD binding.

Results: DFS performed at three different pulling rates (14000, 42000, and 70000) pN/s) yielded rupture forces of 77, 86 and 88 pN; Bell model analysis yielded a dissociation constant, k_{off} ~ 0.03 sec^{-1} and rupture distance x_{-1}~ 0.6 nm. Excess cHArGD in solution dramatically reduced the rupture